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10/045,018	01/15/2002	Eduardo N. Mitrani	01/22527	8957

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EXAMINER	
BERTOGLIO, VALARIE E	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 06/16/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/045,018	MITRANI, EDUARDO N.	
	Examiner	Art Unit	
	Valarie Bertoglio	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 April 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-33* is/are pending in the application.

4a) Of the above claim(s) *1-17 and 29-33* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01/15/2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *05/02*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Election/Restrictions

Applicant's election without traverse of Group IV, claims 18-28 in the election received 04/19/2004 is acknowledged.

Claims 1-17 and 29-33 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Specification

The disclosure is objected to because of the following informalities:

The specification refers to Roza mice on page 38, line 17 and page 39, line 5. It cannot be determined if these are the same mice as the tg-ROSA mice on page 28, line 4 or if they are some other strain of mice.

Appropriate correction is required.

Claim Rejections - 35 USC § 112-1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 18-28 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of generating an artificial micro-organ using a devitalized, acellular three dimensional scaffold and seeding the scaffoldwith stem cells, does not reasonably provide enablement for a method of generating an artificial micro-organ using a synthetic, acellular scaffold and seeding the scaffold with differentiated cells. The specification does not

enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims are drawn to generating an artificial micro-organ by providing an acellular three-dimensional scaffold and seedting the scaffold with cells. Claims 19-22 limit the cell population used in seeding the scaffold. Claims 23 and 24 limit the cells to genetically transformed cells. Claims 25-27 add the method step of generating the acellular three-dimensional scaffold from a micro-organ. Claim 28 specifies that the cells seeded on the microorgan derived scaffold are derived from the same source as the micro-organ used to generate the scaffold.

The specification teaches generating micro-organs from whole organs isolated from a live animal and generating an acellular scaffold from the microorgan by removing the cells from the micro-organ. The specification teaches seeding the devitalized acellular scaffold with stem cells to generate a new micro-organ.

The breadth of claims 18-24 encompass synthetic acellular scaffolds such as a collagen matrix or polyglycolic acid fiber matrix. The specification fails to enable generating a micro-organ using a synthetic scaffold. The specification teaches generating a micro-organ using a scaffold generated by decellularizing a micro-organ. The resulting scaffold is acellular but retains components of the extra-cellular matrix deposited by the cells that once comprised the organ or micro-organ. The specification does not teach how to make a synthetic acellular scaffold that would have the same properties as that generated from an organ or micro-organ isolated from a live animal. The specification specifically teaches that stem cells seeded onto a devitalized acellular micro-organ scaffold differentiate into cell types specific to the organ from which the scaffold was generated (refer to page 49, paragraphs 1-2). The art at the time of filing

held that the growth and differentiation of cells is enhanced by cell products (refer to Naughton, USPN 5,226,480, column 4, lines 15-21). Accordingly, in using a collagen gel as a three dimensional substrate for cell growth, Naughton inoculated the collagen matrix with stromal cells such as fibroblasts to deposit an extracelllar matrix on the collagen (column 5, lines 15-20, for example). Likewise, Schumacher taught that molecular interactions between neural cells and the extracellular matrix is important in regulating differentiation (2001, Jour Biol Chem, Vol. 276, pages 7337-7345, specifically paragraph bridging col. 1-2, page 7337). The instant specification fails to teach how to make a synthetic scaffold that is acellular that has the properties of a once-cellularized scaffold, capable of enhancing cell growth and differentiation and therefore fails to overcome the well-known state of the art concerning the importance of the complex network of molecular interactions with the extracellular matrix in the growth and differentiation of cells and tissues. It would require undue experimentation for one of skill in the art to determine how to generate and synthetic, acellular scaffold with the proper extracellular matrix environment to support specific growth and differentiation to generate a micro-organ.

The breadth of claims 18 and 23-28 encompass seeding any differentiated cell type onto any acellular three-dimensional scaffold. The specification fails to enable generating micro-organs by seeding an acellular micro-organ scaffold with differentiated cells. The specification at pages 39-40 teaches that stem cells seeded on micro-organ derived scaffolds differentiate according to the source of the micro-organ. Specifically, the specification teaches that pluripotent bone marrow stem cells differentiate into lung alveolar structures and express lung specific genes when seeded on lung devitalized micro-organ scaffolds (page 40, paragraph 2; paragraph bridging pages 40-41). The specification does not teach seeding differentiated cells that are no

longer pluripotent onto micro-organ scaffolds. The state of the art at the time of filing was that differentiated cells have lost pluripotency and therefore the ability to generate multiple cell types (refer to Liu, 2003, Jour Cell Biochem, Vol. 88, pages 29-40, specifically page 29, col. 1, paragraph 1). The specification fails to provide evidence that differentiated cells can dedifferentiate and take on new cell fates to generate a micro-organ when seeded on a devitalized acellular micro-organ scaffold. For example, it cannot be determined how to generate a liver micro-organ using a differentiated skin cells by seeding the skin cells on a liver micro-organ scaffold comprising extracellular matrix molecules associated with liver as it was not know in the art how to turn a skin cell into a pluripotent liver cell capable of giving rise to multiple liver cell types and the specification gives no guidance with respect to dedifferentiation of differentiated cells using an acellular scaffold.

Claim Rejections - 35 USC § 112-2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 18-28 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 18 is unclear because the specification does not clearly set forth the metes and bounds of the term "microorgan". The specification defines a microorgan as an "organ portion of unique characters" (page 4, line 18). The specification fails to set forth a concrete definition of the term micro-organ. Claims 19-28 depend from claim 18.

Claim 18 is unclear because the metes and bounds of the term "repopulate" is unclear. It is unclear how many cells need to be present to constitute repopulation of the scaffold. Claims 19-28 depend from claim 18.

Claim 28 recites the limitation "said stem cells" in claim 25. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1) Claims 18-24 are rejected under 35 U.S.C. 102(b) as being anticipated by Vacanti (1999, USPN 5,855,610).

Claim 18 is drawn to a method of generating an artificial micro-organ comprising providing an acellular three dimensional scaffold wherein no cell is more than about 225 micrometers from cells position at a nearest surface of the scaffold and seeding the scaffold with cells. Claims 19 and 20 limit the cells to adult stem cells. Claim 21 limits the cells to embryonic stem cells. Claim 22 limits the cells to a mixed population of stem cells, progenitor cells and differentiated cells. Claims 23 and 24 limit the cells to genetically transformed cells.

Vacanti (1999, USPN 5,855,610) taught seeding a synthetic polyglycolic acid fiber based matrix with fibroblast and endothelial cells. The synthetic polyglycolic acid fiber based matrix is

an acellular scaffold that has three dimensions (col. 3, lines 12-14). Vacanti taught using normal or genetically engineered cells (col. 6, lines 43-44) and differentiated cells as well as adult and embryonic stem cells (column 6, lines 50-53). Vacanti did not teach explicitly that the cell scaffold should be constructed that the cells positioned deepest should be not more than 225 micrometers away from cells positioned at a nearest surface on the scaffold. However, Vacanti did teach that a problem inherent in other scaffolds for tissue generation that the cells in the interior of thick collagen matrix fail to survive (col. 1, lines 18-20) and that a large surface area to volume ratio is necessary to allow for adequate diffusion of gases and nutrients in the absence of vascularization (col. 1, lines 37-46). Vacanti taught that the scaffold determines the limits of tissue growth and that cells implanted on the matrix do not proliferate beyond the matrix. Vacanti taught that the fibrous structure of the polymer is such that free diffusion and nutrients and gases occurs with interstitial spacing and interconnected pores in the range of 100 to 300 microns (col. 3, lines 41-60). With the interstitial spacing of 300 microns any cell along the pore being considered to be on the surface of the matrix then no cell would be more than 150 microns from another cell along a pore, falling within the limitations of the claim. Furthermore, instant specification teaches that cells in vivo are positioned no more than 225-300 microns from the nearest blood vessel and that micro-organs in culture have no cells positioned more than 225-300 microns from growth medium (page 5, lines 8-17). Therefore, it is not apparent that the limitation of a maximum of about 225 microns as claimed would differ from or provide any advantage over the 300 micron interstitial spacing limit taught by Vacanti.

Therefore, Vacanti satisfies all the limitations of claims 18-24.

2) Claim 18 is rejected under 35 U.S.C. 102(b) as being anticipated by Vacanti (1998, USPN 5,770,417).

Claim 18 is drawn to a method of generating an artificial micro-organ comprising providing an acellular three dimensional scaffold wherein no cell is more than about 225 micrometers from cells position at a nearest surface of the scaffold and seeding the scaffold with cells.

Vacanti taught seeding a synthetic matrix with various cell types (col. 3, lines 35-50, column 4, lines 8-18). Vacanti taught that the matrix should be such that the maximum diffusion over which nutrients and gases must occur through a mass of cells attached to the fibers is between 200 and 300 microns (Abstract and claim 1) which differs slightly from the maximum 225 microns of claim 18. The instant specification teaches that cells in vivo are positioned no more than 225-300 microns from the nearest blood vessel and that micro-organs in culture have no cells positioned more than 225-300 microns from growth medium (page 5, lines 8-17). Therefore, it is not apparent that the limitation of a maximum of about 225 microns as claimed would differ from or provide any advantage over the 300 micron limit taught by Vacanti.

Therefore, Vacanti satisfies all the limitations of claim 18.

3) Claims 18-20,22-28 are rejected under 35 U.S.C. 102(b) as being anticipated by Riviere (1995, PNAS, Vol. 92, pages 3733-6737).

Claim 18 is drawn to a method of generating an artificial micro-organ comrising providing an acellular three dimensional scaffold wherein no cell is more than about 225 micrometers from cells positioned at a nearest surface of the scaffold and seeding the scaffold

with cells. Claims 19 and 20 limit the cells to adult stem cells. Claim 22 limits the cells to a mixed population of stem cells, progenitor cells and differentiated cells. Claims 23 and 24 limit the cells to genetically transformed cells. Claims 25-28 limit the method to generating the scaffold from a micro-organ with claim 28 further limiting the stem cells to progenitor cells derived from the same source as the micro-organ.

Riviere taught transplanting bone marrow cells infected with virus encoding human adnosine deaminase, which is an exogenous polypeptide, into a lethally irradiated mouse. The lethal irradiation kills cells in the bone marrow of the mouse such that an acellular scaffold comprising extracellular matrix remains. The bone marrow is porous and vascularized such that no cell is more than 225 micrometers from cells nearest the surface, because, as taught by the specification no cell is positioned more than 225-300 microns from a nearest blood vessel, which represents a surface of the scaffold. Transplanting bone marrow cells into the irradiated mouse constitutes seeding the scaffold. The bone marrow of the mouse represents a micro-organ as defined by the specification as the specification requires that a micro-organ be of sufficient size to maintain micro-architecture of the organ and be sufficiently small that efficient nutrients and waste diffuse with the growth medium in which they are kept. Accordingly, in vivo, nutrients and waste diffuses in and out of capillaries, which supply blood as a growth medium to the cells. Finally, bone marrow comprises pluripotent stem cells as well as more differentiated cells.

Therefore, Riviere satisfies all the limitations of the claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1) Claims 18-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mitchell (August 2000, US2002/0115208) in view of Vacanti (USPN 5,770,417) or Vacanti (USPN 5,855,610).

Claim 18 is drawn to a method of generating an artificial micro-organ comprising providing an acellular three dimensional scaffold wherein no cell is more than about 225 micrometers from cells positioned at a nearest surface of the scaffold and seeding the scaffold with cells. Claims 19 and 20 limit the cells to adult stem cells. Claim 21 limits the cells to embryonic stem cells. Claim 22 limits the cells to a mixed population of stem cells, progenitor cells and differentiated cells. Claims 23 and 24 limit the cells to genetically transformed cells.

Mitchell taught decellularizing a three dimensional tissue and seeding the remaining matrix with cells (paragraphs 0014, 0017, 0020-0022), including genetically engineered cells (paragraph 0130). Mitchell taught that mixtures of cell types as well as precursor cells, such as stem cells could be used to seed the matrix (paragraph 0022 and 0046). Mitchell did not teach the size of the matrix scaffold as set forth by claim 18 of the instant invention.

However, as set forth above, Vacanti taught that a problem inherent in other scaffolds for tissue generation that the cells in the interior of thick collagen matrix fail to survive ('610, col. 1, lines 18-20) and that a large surface area to volume ratio is necessary to allow for adequate diffusion of gases and nutrients in the absence of vascularization ('610 col. 1, lines 37-46).

Vacanti taught that the scaffold determines the limits of tissue growth and that cells implanted on the matrix do not proliferate beyond the matrix. Vacanti ('417 and '610) taught size limitations on the order of that of the instant claims that are within the limits of diffusion, specifically between 100 and 300 microns ('417).

Accordingly, it would have been obvious for one of ordinary skill in the art at the time the claimed invention was made, to use a decellularized tissue scaffold and seed the scaffold with genetically engineered cells as taught by Mitchell using size restrictions as taught by Vacanti. One of ordinary skill in the art would have been sufficiently motivated to generate tissues in vitro using the size limitations taught by Vacanti because by keeping the engineered tissue within the limits of diffusion, one would increase the likelihood of in vitro tissue survival prior to vascularization following in vivo transplant of the engineered tissue (refer to Vacanti, '610, col. 1, lines 17-20 and 36-46).

Thus, the claimed invention is clearly *prima facie* obvious in the absence of evidence to the contrary.

2) Claims 18 and 23-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bruchman (March 1999, US 5,879,383) in view of Vacanti (USPN 5,770,417) or Vacanti (USPN 5,855,610).

Claim 18 is drawn to a method of generating an artificial micro-organ comprising providing an acellular three dimensional scaffold wherein no cell is more than about 225 micrometers from cells position at a nearest surface of the scaffold and seeding the scaffold with

cells. Claims 23 and 24 limit the cells to genetically transformed cells. Claims 25 and 26 limit the source of the scaffold to that generated from a microorgan.

Bruchman taught making a decellularized subendothelila vascular tissue substrate seeded with endoothelial cells (col. 5, lines 17-20; col. 5, line 31; col. 8, lines 21-25). Bruchman generated the substrate using a vessel segment which qualifies as a microorgan as set forth by the specification because the microarchitecture is preserved and nutrients and wastes can be exchanged in culture (col. 8, lines 1-20). Bruchman did not teach the size of the matrix scaffold as set forth by claim 18 of the instant invention.

However, as set forth above, Vacanti taught that a problem inherent in other scaffolds for tissue generation that the cells in the interior of thick collagen matrix fail to survive ('610, col. 1, lines 18-20) and that a large surface area to volume ratio is necessary to allow for adequate diffusion of gases and nutrients in the absence of vascularization ('610 col. 1, lines 37-46). Vacanti taught that the scaffold determines the limits of tissue growth and that cells implanted on the matrix do not proliferate beyond the matrix. Vacanti ('417 and '610) taught size limitations on the order of that of the instant claims that are within the limits of diffusion, specifically between 100 and 300 microns ('417).

Accordingly, it would have been obvious for one of ordinary skill in the art at the time the claimed invention was made, to use a decellularized tissue scaffold and seed the scaffold with endothelial cells as taught by Bruchman using size restrictions as taught by Vacanti. One of ordinary skill in the art would have been sufficiently motivated to generate tissues in vitro using the size limitations taught by Vacanti because by keeping the engineered tissue within the limits of diffusion, one would increase the likelihood of in vitro tissue survival prior to vascularization

upon in vivo transplant of the engineered tissue (refer to Vacanti, '610, col. 1, lines 17-20 and 36-46).

Thus, the claimed invention is clearly *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on (571) 272-0804. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Deborah Crouch

Valarie Bertoglio
Examiner
Art Unit 1632

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